STUDIES IN THE GENUS GYMNOSPORANGIUM—III. THE ORIGIN OF THE TELEUTOSPORE

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Reprinted from Mycologia, Vol. X, No. 4, July, 1918

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B. O. DODGE

(WITH PLATES 9–11)

The manner in which the teleutospores are formed in the rusts has been described by a number of authors, but our knowledge of the origin and development of the teleutospore in *Gymnosporangium* is based mainly on the work of Sappin-Trouffy,[1] Blackman,[2] and Reed and Crabill.[3] Blackman makes the following statement regarding the origin of the spores in *G. clavariaeforme:* "The teleutospores of this form are not borne on the mycelium but arise from comparatively rectangular cells which form a close-set layer on the surface of the mycelium at the points where the teleutospores are developed. . . . They are similar to the teleutospore-bearing cells described by Sappin-Trouffy for *G. Sabinae.* . . . Each of these cells gives origin to a number (not more than three or four) of narrow outgrowths which develop into the stalked two-celled teleutospores."

Reed and Crabill find that in *G. macropus* the teleutospore is formed by the budding of the upper cell of the pseudoparenchyma. "A layer of erect rectangular cells arises from this mycelial mass just beneath and perpendicular to the cortex. These cells elongate and their tips take on gradually the characters of the incipent teleutospores."

Weimer[4] has studied spore development in *G. macropus* and

[1] Sappin-Trouffy, P. Recherches histologiques sur la famille des Urédinées. La Botaniste 5: 59–244, *f. 1–60.* 1896.

[2] Blackman, V. H. On the fertilization, alternation of generations and general cytology of the Uredineae. Ann. Bot. 18: 323–373. *pl. 21–24.* 1904.

[3] Reed, H. S., and Crabill, C. H. The cedar rust diseases caused by *Gymnosporangium juniperi-virginianae* Schw. Virginia Agr. Exp. Sta. Tech. Bull. 9: 1–106. *f. 1–23.* My 1915.

[4] Weimer, J. L. Three cedar rust fungi, their life histories and the diseases they produce. Cornell Agr. Exp. Sta. Bull. 390: 509–549. *f. 136–157.* My 1917.

G. globosum and apparently agrees with Reed and Crabill regarding the first species. "From these stromatic layers the telispore stalks arise." Of *G. globosum* he says: "The telial horns are developed from a stromatic layer in the same manner as are those of *G. juniperi-virginianae*." Weimer's figure 156 shows a mature sorus of *G. clavipes*. At the margin teleutospores are figured arising directly from the outermost cells of the pseudoparenchyma.

If we use the term basal cell in the usual sense, that is, to designate the cell from which various spore forms take their origin, then, according to the authors cited above, the basal cell in the teleutospore sorus is the upper or terminal cell of the pseudoparenchyma or stromatic mass. The writer[5] has recently described the origin of the teleutospore in *G. transformans* and *G. fraternum* and has since studied this question in *G. macropus, G. globosum, G. clavariaeforme* and *G. nidus-avis*. In all of these species at least the true basal cell is not the upper cell in the chain but it is the penultimate cell from which the teleutospore arises by budding. In *G. transformans* and *G. fraternum* the upper cells increase in size considerably, lose their cytoplasm and nuclei by degeneration, and become mere bladdery sacs several times their original length, so that the epidermis is raised further and finally broken open. The penultimate cell then grows out through or between the buffer cells, forming a narrow bud from which the teleutospore is formed by further growth. In *G. transformans* the buds seem to grow up very quickly so that the formation and degeneration of the terminal cells may be overlooked. In *G. fraternum*, however, the buffer cells form a striking palisade layer which frequently extends clear across the sorus primordium.

MATERIAL AND METHODS

Some of the material used in these studies was obtained from plants artificially infected in the greenhouse. In most of the work material from naturally infected plants was also studied. It has

5 Dodge, B. O. Studies in the genus *Gynosporangium*—I. Notes on the distribution of the mycelium, buffer cells and the germination of the aecidiospore. Brooklyn Bot. Gard. Mem. 1: 128–140, *pl. 1* × *f. 1–5*. My 1918.

been found that these rusts developed perfectly normally on plants kept in the greenhouse during the summer and placed in cold frames over winter. Very good results have been obtained by fixing extremely thin free-hand sections of material in Flemming's weaker fluid. It is necessary to orient the pieces accurately in order to avoid cutting oblique microtome sections of the buffer cells and teleutospore buds. Flemming's triple stain was used, and when the Orange G in concentrated aqueous solution is employed the buffer cells can be seen very distinctly; whereas if the other colors predominate, the walls of these large empty cells do not show up conspicuously. Sections were cut 10 μ thick.

GYMNOSPORANGIUM MACROPUS

Cedar apples were obtained from plants growing at Cold Spring Harbor, N. Y., November 30, 1917. The cedars were potted and some were placed in cold frames, others were kept in the greenhouse and two were planted in the garden. Galls from artificially infected cedars were also studied and R. C. Faulwetter sent me a large collection from Clemson College, S. C., in January.

Reed and Crabill (l. c.) describe in considerable detail the growth of the galls caused by this rust. They also studied the growth and appearance of the hyphae in their relation to cells and tissues of the host, and followed the development of the sorus from the early stages. My attention has been focused particularly on the method of the origin of the teleutospore and it is on this question in the main that I am unable to agree with Reed and Crabill. One or two other points may be noted, however. These authors had some difficulty in staining the mycelium to show cross-walls and nuclei of haustoria distinctly. I find that the septa appear very plainly in my preparations and the haustoria take a very delicate stain showing the single nucleus in each, or two nuclei when two are present. Neither do I find that the cortical layer is noticeably thicker over the pits in the galls at points where sori are to appear. Weimer believes that the formation of these pits is due to the inhibition of the growth of certain parenchyma cells by the fungus present, while at other points the cells continue to multiply so that depressions result. I find that the

parenchyma cells in the region between the pits are larger than are those beneath the depressions. This might bring about the formation of the pits. Pits are not formed in galls caused by *G. globosum,* and in most species of this genus the host cells with which the hyphae are associated are usually hypertrophied. In some cases pits are formed in galls before there is any massing of the mycelium in preparation for the formation of a sorus, and I have not noticed any great destruction or crushing of parenchyma cells of the host in early stages of sorus formation as stated by Reed and Crabill.

Hyphae were quite commonly present in the parenchyma of all parts of the galls fixed November 30, but they were in much greater abundance in the region beneath the depressions. In the least matured specimens the hyphae had just begun to push in between the outer and smaller parenchyma cells directly beneath the central part of a depression. Later stages show that these hyphae begin to branch (Pl. 9, Fig. 1) and become fairly definitely directed. The cells are slightly larger than the cells of the vegetative hyphae. Their nuclei are very conspicuous, both visible, lying on the long axis of the cell. Although the ends of the branches do not appear to be pushing strongly against the cork layer above, a small space now exists between the cork and the parenchyma of the host. As these hyphae branch and new cells are added, a quite definite loose palisade of radially directed hyphae is formed, the cells of which are somewhat longer than broad. The hyphal mass becomes more compact so that a pseudoparenchyma is formed with cells more or less rectangular or polyhedral in shape. The two nuclei now occupy various positions in the cells (Pl. 9, Figs. 2, 3). The upper cells grow against the cork layers and become somewhat flattened but they are usually slightly longer than the cells beneath. They soon begin to swell or elongate, their cytoplasm becomes more vacuolate and the nuclei take the safranin stain, while the nuclei of all the cells below take the gentian violet. The buffer cells finally become two or three times their original length and contain only a thin watery substance that is faintly colored with orange G. Their walls become thinner and thinner and finally disappear altogether. The

cork cells have been pushed up, crushed in or broken down. This disorganization may not be entirely due to pressure. The fungus evidently brings about some chemical changes in the suberized cells. After the buffer cells have lost their contents the cells below bud out, their nuclei move up to the base of the buds and divide. There are now two nuclei in the bud and two in the basal cell. A septum is formed at once. The young binucleated teleutospore bud grows comparatively slowly so that these stages are fairly abundant at the center of the young sorus (Pl. 9, Fig. 3). Buds may push out of the side of a basal cell (Pl. 9, Fig. 2) and later three or four buds may be formed from one cell. The cork layer is lifted up and broken open first at the central part of the pit. Mature spores are present when this slight swelling or blistering is first noticeable (Pl. 9, Fig. 4). The cells of the lower portion of the stromatic mass do not stain very readily, so that their nuclei do not appear distinctly. Reed and Crabill's diagram (l. c., Fig. 7) of a sorus, while it perhaps exaggerates somewhat, brings out the point that the spores push up rapidly from a region in the center of the depression, and further development peripherally is considerably retarded. The size of the papilla at the center of a depression in the gall determines roughly the diameter of the sorus as it emerges. At the center of the blister the cork cells, especially the innermost cells, are considerably disorganized and crushed in, and nearly mature spores have been formed. Near the margin many long two-nucleated spore buds are present. Buffer cells have disappeared in the whole region beneath the blister, but at its margin and immediately beyond for a short distance a different picture is presented. The cork cells have not been crushed in or lifted up so conspicuously, and buffer cells are plainly visible, fully elongated, and short buds, some without nuclei, are growing up from the basal cells. Figure 4 in plate 9 shows a portion of a young sorus at a time when the papilla is plainly visible but before the cork has been ruptured. The conditions near the margin of the blister are shown at the right in the figure. The sorus primordium ends rather abruptly just beyond. The diameter of the raised portion is about one half of the entire depression in this section. Figures 1 to 4 show the

general features of small portions at the center of young sori. Various stages in the degeneration of the terminal cells will be described in connection with the discussion of the next species, where the conditions are practically the same.

GYMNOSPORANGIUM GLOBOSUM

The best material was obtained at Cold Spring Harbor, November 30. The location of the primordia could not be determined definitely on this date, as the sori had not even begun to form. The rust was therefore allowed to develop further in the greenhouse until December 16. The young sori could then be located by the appearance of mound-like swellings over which the cork layer was tightly stretched. The covering was as yet unbroken. Sections of such a swelling showed a broadly elliptical sorus primordium, at the center of which teleutospore buds were plainly visible. Sori began to break through the surface about ten days later and by the middle of January the large telia had developed quite normally.

The cork layer covering the galls is somewhat thicker than we find in the galls caused by *G. macropus*. There are usually five to eight layers of irregular cork cells and the outer ones may be more strongly suberized. The parenchyma cells are well supplied with starch, and haustoria are present, although not in considerable numbers. There is usually a single nucleus in each haustorium. The earlier stages in the formation of the sorus are similar to those described in connection with the preceding species. There is a more pronounced massing of the hyphae and the pseudoparenchyma is more compact (Plate 10, Fig. 5).

In *G. globosum* the development of the sorus is evenly progressive. At the margin the hyphae are crowded in between the outer parenchyma cells, lifting up the cork layer slightly. Further in the pseudoparenchyma is well formed with terminal cells pushed up squarely against the cork layer. Figures 9 and 10 in plate 11 were drawn from different portions of a section through a sorus. Figure 9 shows the condition at the margin, which is abnormally high in this section, due to an irregularity in the gall. Figure 10 represents a more mature condition toward the center.

A great many different stages can be found in the two regions figured. The sorus has about reached its maximum width and very little marginal extension will occur. In the region at the left between *a* and *b* the growth of the hyphal mass has not been completed. At *b* the terminal cell appears to have begun to degenerate, to judge by the condition of its nuclei. The subterminal cell of this hypha is rather long for a basal cell and no doubt another cell will be cut off. From *c* to *d* terminal growth has been completed, so that the buffer cells can be recognized; they have begun to elongate and their nuclei are seen in the early stages of degeneration. In one cell the nuclei are breaking down. At *m* (below) the nuclei are degenerating without swelling. At *o*, *q* and *r* the degenerating nuclei look like poorly fixed division figures. There is a depression in the pseudoparenchyma at *f*, as though the cork cell above offered considerable resistance. The basal cells here are at a lower level. Growth certainly was not as vigorous at this point for some reason. At *q* and *r* very long buffer cells still persist in a region where spores are being matured. The whole primordium is quite a compact mass of cells wedged up under the cork.

Stages similar to those shown from *d* to *g* in the upper figure were found for a considerable distance along the section. A region in which most of the basal cells have begun to bud out is shown in the lower figure. At *h* the bud has pushed into the empty terminal cell. The nuclei have not yet begun to divide. At *p* two adjacent cells have somewhat longer buds and the nuclei are dividing conjugately. At *k* nuclear division has been completed and the four nuclei appear to lie well up in the tube. This bud is not within the buffer cell but is bent downward so that the septum if formed is not visible. At *i* there are two buds from the same cell, one of them is growing out between two buffer cells. Several other similar stages can be seen in the figure. The two nuclei are dividing in an older bud in the region near *s*, where two-celled buds and two-celled spores are also shown. The nuclei in the cells of the larger spore are just fusing. The cork layers above are not yet completely broken open at this point. Undoubtedly the further elongation of spore stalks will be a factor in completing the rupture.

With the gradual increase in size of the cells as we pass from the margin we find a corresponding enlargement of the nuclei, although there is considerable variation in this respect. At the margin the nuclei are small and spherical or elliptical in outline. Pear-shaped nuclei or nuclei with beak-like extensions are often found in the older cells. The terminal cells do not necessarily form a perfectly even layer. Some cells extend much beyond others. This is due to the inequality in the rate of growth, and perhaps to the fact that some of the cork cells give way more easily. The gall is often irregular in outline.

Having shown that the leaf forms of *Gymnosporangium* on *Chamaecyparis,* and the two cedar apple rusts on the red cedar develop their teleutospores from subterminal basal cells, we may next consider *G. clavariaeforme* which ordinarily inhabits the stem of the common juniper.

GYMNOSPORANGIUM CLAVARIAEFORME

Artificially infected junipers furnished the basis for the study of *G. clavariaeforme.* Sori in several stages of development were found January 17 in the stems of small plants ten days after they had been taken from the cold frame. The cork covering these young stems was not thick enough to cause any serious trouble in fixing or sectioning. Sappin-Trouffy and Blackman studied the origin of the teleutospore in this species and found as previously noted that the terminal cells of the hyphal mass give rise directly to buds from which spores will develop. A brief examination of my material was sufficient to show that such is not the case. The method of origin here is just the same as it is in the species which I have discussed above. The sori on the stem present very much the same picture as do those of *G. globosum* and it is unnecessary to go into details further than refer to figures 6 and 7 in plate 10 which show small portions of a young sorus, one near the margin, the other toward the center. In the first figure we see that the hyphae have pushed up against the cork layers, the end cells have become flattened and rectangular in shape. There is then the same characteristic elongation and degeneration of these terminal cells, and buds grow out from the

subterminal cells and form the teleutospores. Some of the young buds coming against the cork cells are bent sidewise or even downward because the cork layer has not as yet been sufficiently raised (Fig. 7). This figure shows three buds in which the two nuclei are dividing. As Blackman has published good figures of the later stages, no further study was made of spore formation.

GYMNOSPORANGIUM NIDUS-AVIS

The three stem forms, *G. nidus-avis, G. clavariaeforme,* and *G. clavipes* sometimes develop sori on the blades of leaves. A comparison of such leaf sori in the three species discloses interesting differences in the way in which the primordium develops. The first two species develop mycelium in all parts of the leaf, the hyphae ramifying among the wood cells. *G. clavariaeforme* causes a considerable enlargement or hyperplasia, *G. nidus-avis* induces some enlargement of individual cells of the leaf, but the total effect is not very striking. *G. clavipes* forms a sorus which is very superficial and the mycelium is very much localized.

The material that was used in the study of *G. nidus-avis* was taken from artificially infected plants, nos. 929 and 609, and from naturally infected plants where the sori develop on rather conspicuous gall-like mounds beneath the cork. The sorus is formed in much the same way as we found in *G. clavariaeforme.* The cork may be somewhat thicker in the specimens examined and the pseudoparenchyma even more extensively developed and compact. Figure 8, Pl. 10, shows the marginal region of a sorus, the main part of which had already developed mature spores. The terminal cells begin to degenerate before they elongate very much. Their nuclei are often smaller than are those of the basal cells at this stage. All cells of the primordium are more or less rectangular and are closely crowded together. At the right in the figure one basal cell has budded out laterally and another has pushed into the buffer cell. Other stages are similar to those described in the preceding species.

CONCLUSIONS

The origin of the teleutospores from the subterminal cells of the tissue composing the primordium has been shown to be a very

common method in this genus of the rusts. The hosts attacked by the six species studied represent three species and two genera of conifers. The epidermis of the leaves and young stems is rather heavily cutinized, and the galls or stems upon which the sori of some of the species develop are covered with layers of cork of considerable thickness. After the cork (or epidermis) has been lifted up or broken open and the pressure on the marginal hyphal mass has been removed we might expect to find that the later-formed spores (at the margin) arise directly from the terminal cells. If the terminal cell becomes disorganized in response to a particular need for some sort of space-making unit it would not be necessary for every terminal cell in the primordium to degenerate. It is sometimes difficult to ascertain just how the spores are formed at the margin of a nearly full-grown sorus. I have as yet found no evidence that they arise at any time from terminal cells. It is not expected, of course, that this rule will apply to all other species of *Gymnosporangium.*

If the terminal cell represents a morphological unit in the primordia of the rusts, a unit not a basal cell, but one having either the space-making function or some unknown function we ought to find such units in other genera. The presence of sterile tissue in the aecidium primordium in addition to the sterile cells above the gametic cells has been noted by those who have studied this spore form. The morphological or phylogenetic significance of these peculiar cells certainly has not been overemphasized, and it would be interesting to know just to what extent space-making requirements could lead to degeneration of tissue in the primordia of all spore forms, uredo sori and spermogonia included.

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EXPLANATION OF PLATES

The drawings were made with the aid of the camera lucida. Zeiss No. 4 eyepiece and 1/12th oil immersion lens were used in making Figs. 1, 2, 4–8. No. 5 eyepiece was used for Figs. 3, 9 and 10. Reduction about one fourth for Plates 10 and 11 and about one third for Plate 9.

PLATE 9, *Gymnosporangium macropus*

Fig. 1. Beginning of a sorus primordium at the center of a depression or pit in the gall.

Fig. 2. Older stage. About one third of the primordium is shown here. Further extension is being made at the left. Degenerating terminal cells are shown at the center, and spore buds are pushing into or between buffer cells at the right. The nuclei are just dividing in one bud. The walls of the overlying cork cells at the right are being pushed in.

Fig. 3. At the center of another young sorus. Shows that some terminal cells disintegrate more quickly than others. The position of the nuclei in a young bud just prior to division can be seen at the left. The cork cells are very irregular and are somewhat distorted by pressure.

Fig. 4. A portion of an older sorus at the stage when the papilla is quite noticeable. Two cork cells (at the left) are completely broken up and spores are nearly mature. Two or three spores or spore buds arise from each basal cell. No traces of buffer cells can be seen. At the right of the cells bearing two-celled spores there are six or seven basal cells that bear spore buds in which the stalk cell of each has just been cut off. There are one or two buds having three cells. The next region shows two-nucleated buds and other buds just forming. Traces of buffer cell walls are now visible as mere lines. At the right in the figure the degenerating terminal cells are very distinct. This figure shows the conditions near the border of the blister-like swelling. Note the abrupt transition here, practically all stages in spore formation from those in which the terminal cells still possess nuclei to the stages where two-celled spores are completed.

PLATE 10

Fig. 5, *Gymnosporangium globosum*. The central portion of a young primordium. Terminal cells have elongated slightly but have not begun to degenerate. The nuclei are somewhat larger than are those in the primordium of *G. macropus*.

Fig. 6, *Gyymnosporangium clavariaeforme*. The region at the margin of a young sorus at the center of which spores are just beginning to form. The cells from which the spore buds arise are plainly at a lower level than the terminal cells at the left. The host cells contain masses of some deeply staining substance showing degeneration, but their nuclei appear to be quite normal. An haustorium is shown in one cell.

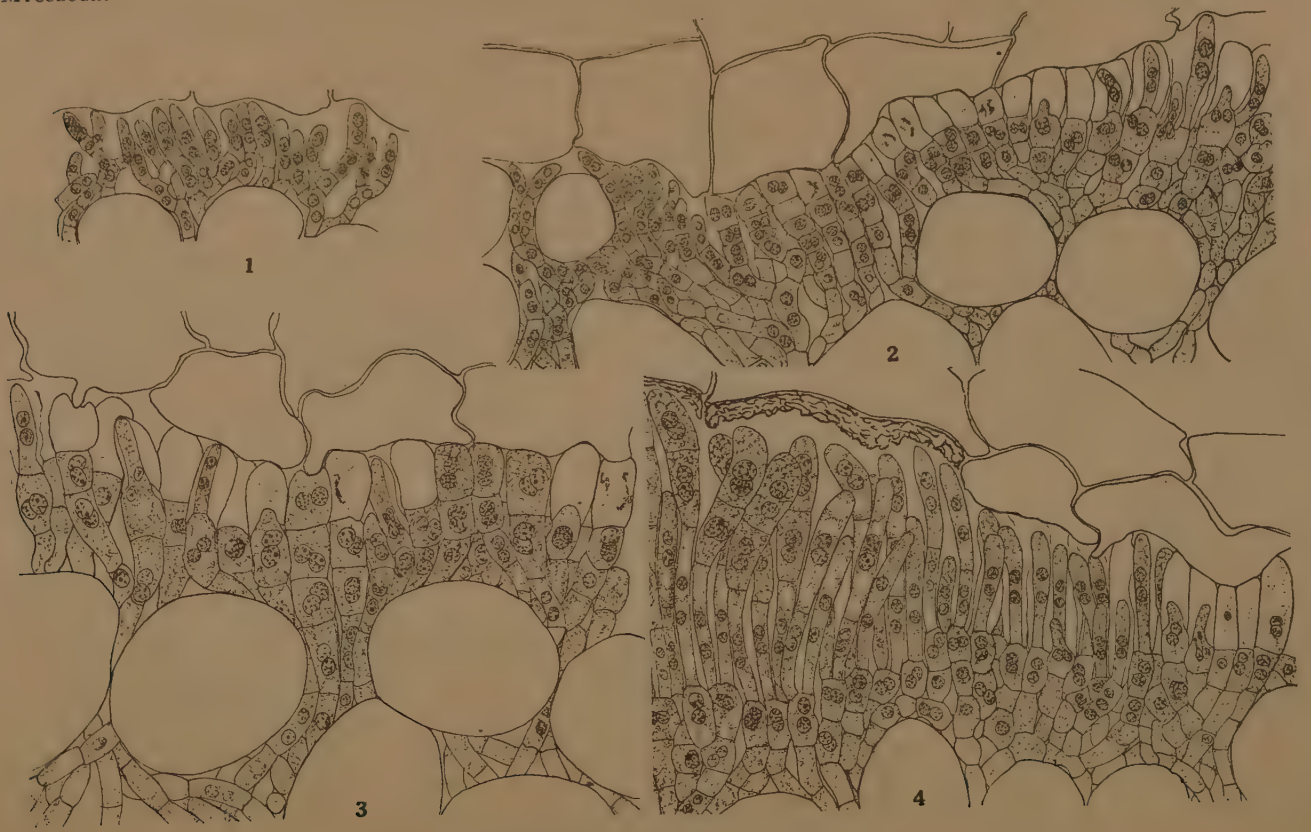
Fig. 7, *Gymnosporangium clavariaeforme*. Region at the center of a younger sorus. In one cell a bud is just forming. Nuclear division is occurring in the buds from three adjacent subterminal cells, one of which also has a bent two-nucleated lateral bud.

Fig. 8, *Gymnosporangium nidus-avis*. Near the margin of a large sorus on a naturally infected plant. Mature spores were present at the center and the cork had just been ruptured. There were about fifteen rows of flattened thick-walled cork cells above the region shown in the figure.

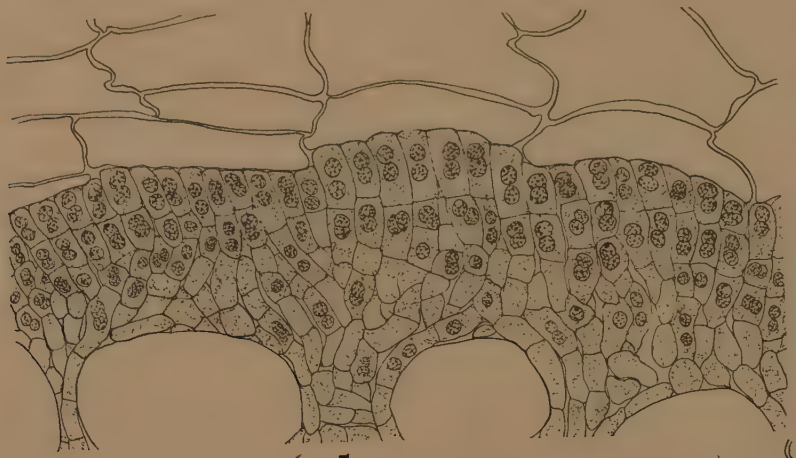
The nuclei of the terminal cells are small, and degeneration is not preceded by such an amount of elongation as occurs in the other species. A lateral bud and an internal spore bud are shown at the right. The nuclei of the cells of the pseudoparenchyma are rather large.

PLATE 11

Figs. 9 and 10, *Gymnosporangium globosum*. The upper figure shows the conditions from the margin of a sorus inward. The lower figure is drawn



GYMNOSPORANGIUM MACROPUS



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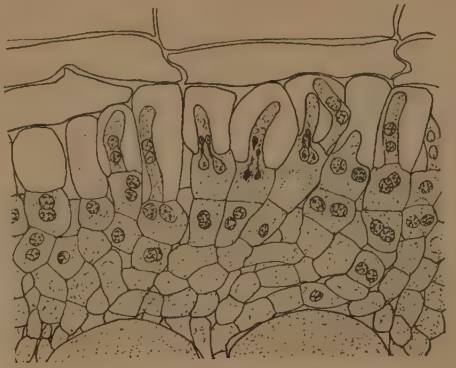

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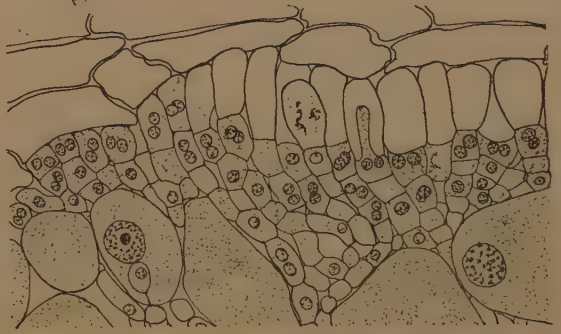

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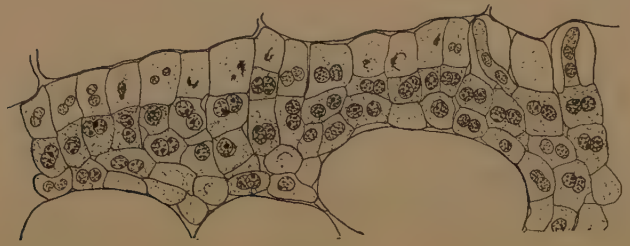

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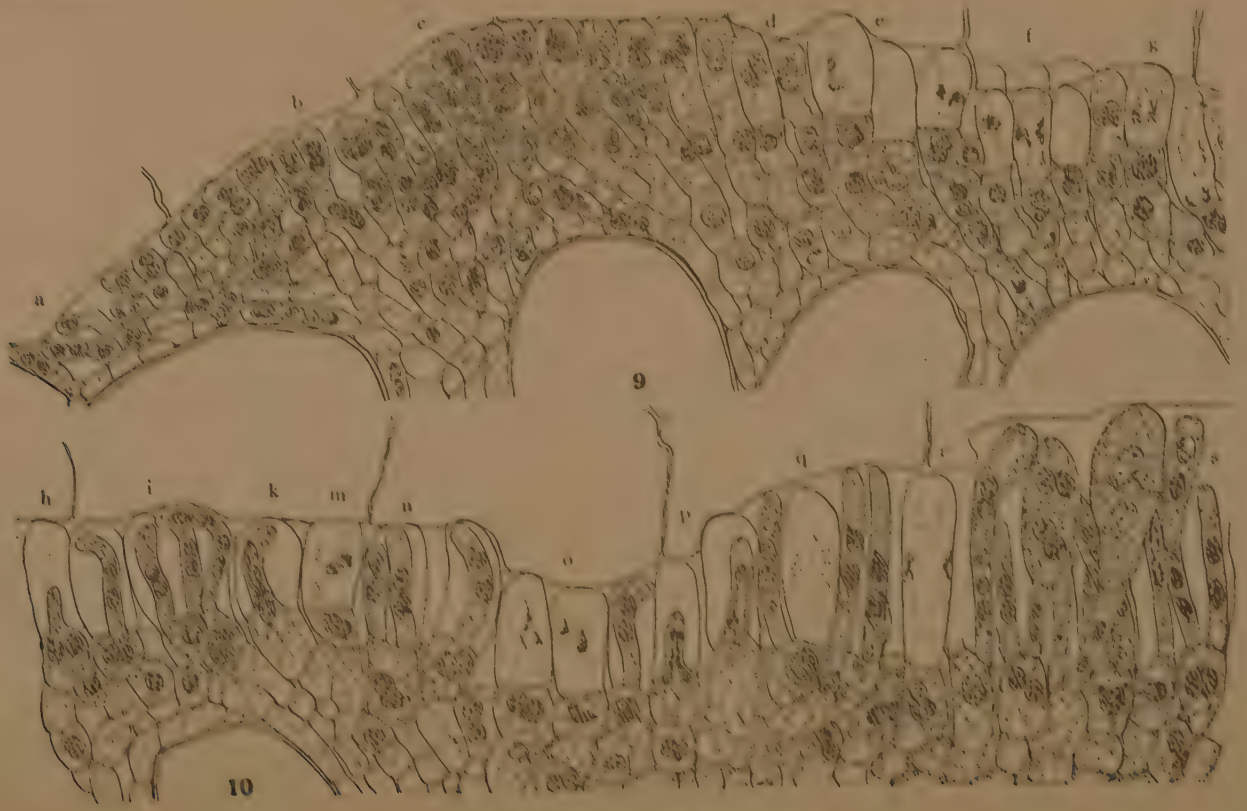
FIG. 5. G. GLOBOSUM. FIGS. 6, 7. G. CLAVARIAEFORME. FIG. 8. G. NIDUS-AVIS



GYMNOSPORANGIUM GLOBOSUM

from the same section but at about the center of the sorus. Between the two parts shown there is a space equivalent to about five or six times the amount shown in the figures. At *a* (Fig. 9) marginal growth is still continuing slowly. The nuclei in the terminal cell at *b* appear to be degenerating. The sorus is abnormally high at *c-d* due to an irregularity in the gall. Here the terminal cells have been cut off and their cytoplasm is becoming more vacuolated; *e*, first buffer cell that has lost both cytoplasm and nuclei; *f*, *g*, a depression in the primordium due to a less vigorous growth of the hyphal mass below; *m*, *o*, *q*, *r*, other terminal cells in various stages of degeneration; *h*, a basal cell just beginning to bud; *p*, conjugate division of the nuclei in young spore buds; *k*, nuclear division completed in a bud that pushes out laterally from the basal cell on its lower side, and the septum, if formed, is not visible because the bud bends down and is not within the terminal cell as it appears in the figure; *i*, *n*, *q*, *r*, two-nucleated buds cut off by septa from their basal cells; *s*, four-nucleated buds, nuclei of a two-nucleated bud dividing, two-celled spores, nuclear fusions, etc.